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WALTER WYMAN, Surgeon-General.

HYGIENIC LABORATORY.—BULLETIN No. 8.

AUGUST, 1902.

Biological & Medical Serials

LABORATORY COURSE IN PATHOLOGY AND BACTERIOLOGY

BY

M. J. ROSENAU,
DIRECTOR OF HYGIENIC LABORATORY.

WASHINGTON:
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1902.

NOTICE TO LIBRARIANS AND BIBLIOGRAPHERS, CONCERNING THE SERIAL PUBLICATIONS OF THIS SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, to be located in Washington, was authorized by act of Congress, March 3, 1901.

By act of Congress, approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

The following *bulletins* [Bull. Nos. 1-7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

No. 3.—Sulphur dioxide as a germicidal agent. By H. D. Geddings.

No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxide. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

The bulletins of the hygienic laboratory will continue in the same numerical order, the present issue being No. 8.

In citing these bulletins, beginning with No. 8, bibliographers and authors are requested to adopt the following abbreviations: Bull. No. ——, Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv., Wash., pp. ——.

A list of the other serial publications, issued by the Service, will be published later.

MAILING LIST.

The Laboratory will enter into exchange of publications with medical and scientific organizations, societies, laboratories, journals, and authors. Its publications will also be sent to nonpublishing societies and individuals in case sufficient reason can be shown why such societies or individuals should receive them. All applications for these publications should be addressed to the "Surgeon-General, U. S. Public Health and Marine-Hospital Service, Washington, D. C."

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LETTER OF TRANSMITTAL.

SIR: I have the honor to transmit herewith a syllabus of the course in pathology and bacteriology given in this laboratory.

This is the completion of the third year in which the course of instruction as outlined in the following pages has been given by the present director of the laboratory to the student-officers of the Service. Advantage has been taken of many practical points gained from actual experience both from the standpoint of the student and that of the instructor, so that the course as now outlined is believed to fairly cover the ground and to require few changes other than those due to advances in our knowledge upon the subjects treated.

The object of the course is to thoroughly equip officers of the Service in the technique of pathology and bacteriology so as to fit them better for the hospital, quarantine, epidemic, and public-health work of the Service. Officers of the Service who have taken the prescribed course are thoroughly prepared to make the scientific diagnoses of plague, cholera, diphtheria, tetanus, tuberculosis, typhoid fever, anthrax, and other communicable diseases, as well as to carry on original investigations for themselves.

The course as outlined requires about one year for its completion. The student-officer is required to work each subject until he has mastered it before going on to the next. Such deliberation permits a thoroughness which is not possible when work is hurried or limited to a short space of time.

The exact sequence as given on the following pages is not strictly adhered to, because it is the desire of the director to make the course as practical as circumstances will permit. Therefore, the study of diphtheria is usually left until the fall of the year or winter, when practical experience may be had with the cases which usually occur in the District. The studies upon vaccinia and smallpox are taken up in the winter, which time is more apt to offer material and cases. Malarial work is of course confined to the summer and fall seasons. In connection with the health department of the District, the work of diagnosing diphtheria from swabs and culture tubes and the administration of antitoxin, both for prophylactic and curative purposes, is carried on by each student-officer. In connection with the studies upon vaccinia, a visit is always made to one of the large vaccine farms, which offers special advantages for observing the process of vaccinating the calves, obtaining the lymph and pulp, glycerinating the virus, and preparing the product for the market. In like manner advantage is taken, as far as it is practicable, of the material in hospitals and other institutions situated in the city of Washington.

It will be noticed that the course apparently deals more with the subject of bacteriology than that of pathology. The latter, however, is not neglected. Owing to circumstances, it is not possible at present to treat pathologic-histology in the same systematic manner as bacteriology. The pathologic-histology of all the infectious processes studied in the laboratory are thoroughly worked. Tumors and the special pathology of the organs are taken up from time to time as the specimens are received. Experience has taught that this is better than to attempt to give a systematic course in general and special pathology, in which branches the student-officer usually comes to the laboratory with a good foundation.

It will also be noticed that considerable importance is given to the subject of disinfections and disinfectants. This arises from the fact that officers of the Service are required not only in their hospital work, but more especially in their quarantine and public health duties, to thoroughly understand the theory and practice of this important subject. Each student-officer is required to master the theory and mechanics of all the important disinfecting apparatus and to make actual tests upon the efficiency and practical value of each one.

The scientific workers in the laboratory meet one evening in each week in order to review the work of the week and to discuss the current literature. The journals that are received are assigned to each student-officer, and it is his duty to present a critical review of important articles which he reads. We have found this an admirable plan for keeping thoroughly abreast with the large amount of literature constantly appearing, as well as a stimulus to thought and work.

After the student-officer has been in the laboratory several months and has thoroughly acquainted himself with the necessary technique, opportunity is given him to assist with the work of the laboratory, and every encouragement is offered to work out original problems of his own.

The classes have been small in number, thus allowing for the individual equation of each student-officer, as well as a maximum of personal attention.

The bacteriologic nomenclature adopted in this bulletin is based upon Migula's recent System der Bakterien, 1900, and I am indebted to Dr. Ch. Wardell Stiles for valuable assistance in determining many points in this difficult and much-neglected subject.

Respectfully, M. J. ROSENAU.

Passed Assistant Surgeon and Director Hygienic Laboratory.

The SURGEON-GENERAL.

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STUDIES FOR MICROSCOPIC PRACTICE—

1. Diatoms.
2. Algae.
3. Protozoa.
4. Starch bodies.
5. Vegetable cells.
6. Cotton fibers.
7. Linen fibers.
8. Silk fibers.
9. Feathers.
10. Hair of man and of lower animals.
11. Scales of butterflies and of other insects.
12. Milk.
13. Air bubbles.
14. Oil drops.
15. Vinegar eels (*Anguillula aceti*).
16. Tube casts.
17. Ciliated epithelium.
18. Crystals of sugar, salt, uric acid, cholesterin, hematin, and various chemicals.
19. Decomposing meat.
20. Hay infusion.
21. Surface waters.

POINTS TO BE OBSERVED IN DESCRIBING AN ORGANISM—

1. Its source.
2. Hanging drop. Form, size, grouping and motility.
3. Spores.
4. Staining peculiarities.
5. Flagella.
6. Appearance of colonies on gelatine, agar, blood-serum, potato, etc.
7. Relation to oxygen.
8. Growth on gelatin.
9. Growth on agar.
10. Growth on blood-serum.
11. Growth on potato.
12. Growth on fluid media:
    - *a.* Bouillon—
        1. Acid.
        2. Neutral.
        3. Alkaline.
    - *b.* Dunham's solution—indol.
13. Milk and litmus milk.
14. Reaction of media best suited to growth.
15. Changes in reaction of media.
16. Gas production :
    - *a.* Glucose bouillon.
    - *b.* Lactose bouillon.
    - *c.* Glucose agar.
    - *d.* Glucose gelatin.
17. Temperature best suited to development.
18. Pathogenicity.
19. Reaction to sera of immune animals.
20. Toxins.

STUDY OF SAPROPHYTES.

BACTERIA.

Genus *Bacillus* Cohn—

1. *Bacillus subtilis* (Ehrenberg, 1833) Cohn, 1872.
2. *Bacillus vulgaris* (Hauser, 1885) Migula, 1900. [*Proteus vulgaris.*]
3. *Bacillus megaterium* de Barry, 1884.
4. *Bacillus prodigiosus* (Ehrenberg, 1839) Flügge, 1886.

Genus *Pseudomonas* Migula—

5. *Pseudomonas fluorescens* Migula, 1900. [*Bacillus fluorescens liquefaciens* Flügge, 1886.]
6. *Pseudomonas aeruginosa* (Schröter, 1872) Migula, 1900. [*Bacterium aeruginosum* Schröter, 1872; *Bacillus pyocyaneus* Gessard, 1882.]

Genus *Planosarcina* Migula—

7. *Planosarcina agilis* (Ali-Cohen, 1889) Migula, 1900. [*Micrococcus agilis* Ali-Cohen, 1889.]

Genus *Sarcina* Goodsir—

8. *Sarcina lutea* Schröter, 1886.

Genus *Microspira* Schröter, *1886*—

9. *Microspira aquatilis* (Günther, 1892) Migula, 1900. [*Vibrio aquatilis* Günther 1892.]

COMMON MOLDS—

Oidium.
Aspergillus.
Penicillium.
Mucor.

YEASTS—

Beer yeast.
Red yeast.
Black yeast.

CLASSIFICATION OF SCHIZOMYCETES—

1. According to some characteristic feature:
    - *a.* Chromogenic.
    - *b.* Photogenic.
    - *c.* Zymogenic.
    - *d.* Saprogenic.
    - *e.* Pyogenic.
    - *f.* Thiogenic.
2. According to morphology:
    - Cocci—
        - *a.* Micrococci.
        - *b.* Diplococci
        - *c.* Streptococci.
        - *d.* Staphylococci.
        - *e.* Tetrads.
        - *f.* Sarcinae.
        - *g.* Merismopedia.
        - *h.* Ascococcus.
    - Bacilli—
        - *a.* Leptothrix.
        - *b.* Cladothrix.
        - *c.* Streptothrix.
    - Spirilla—
        - *a.* Vibrio.
        - *b.* Spirillum.
        - *c.* Spirochæta.
3. According to motion:
    - *a.* Nonmotile.
    - *b.* Motile.
    - *c.* Brownian motion.
4. According to arrangement of flagella:
    - *a.* Monotrichic.
    - *b.* Amphitrichic.
    - *c.* Lephotrichic.
    - *d.* Peritrichic.
5. According to relation to spores:
    1. Nonspore bearing.
    2. Spore bearing—
        - *a.* Endospores.
        - *b.* Arthrospores.

MAKING PLATES.

1. Koch's method—glass plates.
2. Esmarch's roll tubes.
3. Slants of agar and gelatin in test tubes.
4. Petri dishes—
   - Streaking on surface with platinum wire.
   - Daubing with swabs on surface.
   - Mixing while fluid.

OBTAINING PURE CULTURES—

1. By plate method on solid media.
2. By dilution in liquid media.
3. By temperature.
4. Inoculation into animals.
5. Negative plates.
6. Special methods.

OBSERVE GROWTH AND MULTIPLICATION OF BACTERIA IN CELL SLIDE — AGAR METHOD:

1. Division of bacilli.
2. Division of cocci.
3. Sporulation.

STUDY OF BROWNIAN MOVEMENT WITH:

India ink and other substances in hanging drop.

THE PRINCIPLES, CHEMISTRY, AND PRACTICE OF STAINING—

- A. Vegetable stains:
  - 1. Carmine—
    - *a.* Acid.
    - *b.* Alcoholic.
    - *c.* Alum.
    - *d.* Borax.
  - 2. Hematoxylin—
    - *a.* Delafield's hematoxylin.
    - *b.* Alum hematoxylin.
    - *c.* Hemalum (Meyer).
    - *d.* Hematoxylin and eosin.
- B. Aniline stains:
  - 1. Acid stains—
    - *a.* Eosin.
    - *b.* Acid fuchsin.
    - *c.* Orange G.
    - *d.* Soudan.
    - *e.* Picric acid.
  - 2. Basic stains—
    - *a.* Methylene blue (Löffler's solution).
    - *b.* Thionin.
    - *c.* Fuchsin (Carbol-fuchsin).
    - *d.* Gentian violet (Ehrlich's aniline water.)
    - *e.* Methyl violet.
    - *f.* Bleu d' argent.
- C. Gram's method and solution:
- D. Decolorization and differentiation:
    - *a.* Water.
    - *b.* Alcohol.
    - *c.* Aniline oil.
    - *d.* Hydrochlorate of aniline, 2 per cent.
    - *e.* Acids—organic and mineral.
- E. Staining of spores:
  - Möller's method.
- F. Staining of flagella:
    - *a.* Löffler's method.
    - *b.* Victoria blue.
    - *c.* Von Ermengem's method.

PRINCIPLES, CARE, AND USE OF INSTRUMENTS AND APPARATUS—

a. Centrifuge.
b. Crematory.
c. Photographic apparatus.
d. Cleaning glass ware.
e. Microscope.
f. Paraffin bath.
g. Analytical balance.
h. Microtome.
i. Thermostats.
j. Thermo-regulators; electric, mercurial, metallic.
k. Vacuum pumps.
l. Blow pipe.
m. Camera lucida.
n. Spectroscope.
o. X-ray tubes and induction coil.
p. Autoclaves.
q. Dry wall sterilizer.
r. Arnold steam sterilizer.
s. Blood serum sterilizer.
t. Cold chamber.
u. Sterilization of instruments.
v. Sharpening and care of cutting instruments.

OPTIC PRINCIPLES INVOLVED IN MICROSCOPY—

1. Lenses:
   - *a.* Plano-convex.
   - *b.* Biconvex.
   - *c.* Concave.
   - *d.* Biconcave.
   - *e.* Doublets.
   - *f.* Triplets.
   - *g.* Quadruplets.
2. Objectives:
   - *a.* Achromatics.
   - *b.* Apochromatics.
   - *c.* Panchromatics.
3. Oculars:
   - *a.* Huyghenian.
   - *b.* Compensation.
   - *c.* Spectroscopic.
   - *d.* Micrometer:
   - *e.* Projection.
4. Condensers :
   - *a.* Simple—
     1. Biconvex.
     2. Plano-convex.
   - *b.* Abbe—
     - Numerical aperature 1.20.
     - Numerical aperature 1.40.
5. Microphotography and projection :
6. Micrometers :
   - *a.* Stage.
   - *b* Ocular.
   - *c.* Projection.

MEASURING MICROSCOPIC OBJECTS—

1. Eye-piece micrometer.
2. Stage micrometer.
3. Photographic method.

GLASS BLOWING—

1. The blowpipe.
2. Pipettes.
3. Bulbs.
4. Welding.
5. Sealing and opening glass containers.
6. Making test tubes.
7. Making perforated tubes for collodium sacks.

FOLDING PAPER FILTERS.

PREPARING BACTERIOLOGIC FILTERS (PRINCIPLES AND PRACTICE)—

1. Kaolin.
2. Diatomaceous earth.
3. Asbestos.
4. Sand filtration.

PREPARATION AND MOUNTING OF HISTOLOGIC SPECIMENS—

1. Fixation:
   - *a.* Acetic-bichlorid of mercury.
   - *b.* Müllers fluid.
   - *c.* Chrom-acetic acid.
   - *d.* Flemming's solution.
   - *e.* Formaldehyd.
   - *f.* Absolute alcohol.
2. Dehydration:
   - *a.* Alcohol 70 per cent (iodine alcohol if $Hg\ Cl_2$).
   - *b.* Alcohol 85 per cent.
   - *c.* Alcohol 95 per cent.
   - *d.* Absolute alcohol, twice.
3. Clearing:
   - *a.* Xylol.
   - *b.* Chloroform.
   - *c.* Turpentine.
   - *d.* Oil cloves.
   - *e.* Oil bergamot.
   - *f.* Oil origanum.
4. Infiltration and imbedding:
   - *a.* Paraffin.
   - *b.* Celloidin.
5. Sectioning:
   - *a.* Single sections.
   - *b.* Serial sections.
6. Attaching section to slide:
   - *a.* Alcohol 85 per cent.
   - *b.* Water.
   - *c.* Myer's albumen.
   - *d.* Gum Arabic.
   - *e.* Collodium and clove oil.
7. Removal of imbedding material:
   - *a.* Xylol to remove paraffin.
   - *b.* Alcohol and water.
8. Staining:
   - *a.* Nuclear staining.
   - *b.* Protoplasmic.
   - *c.* Regressive.
   - *d.* Gram's.
   - *e.* Bacterial.
   - *f.* Special.
9. Dehydration in absolute alcohol, aniline oil:
10. Clearing:
    - *a.* Xylol.
    - *b.* Turpentine.
    - *c.* Oil origanum.
    - *d.* Oil bergamot.
    - *e.* Oil cloves.
    - *f.* Chloroform.
11. Mounting in xylol balsam:

THE PARAFFIN BATH AND PARAFFIN IMBEDDING—

A. Fixing.
B. Dehydration.
C. Clearing.
D. Imbedding in paraffin.
E. Molding block.
F. Cutting.
G. Method of numbering, registering, and storing.

CELLOIDIN IMBEDDING—

A. Prepare a thin celloidin solution containing 4 per cent celloidin in equal parts of absolute alcohol and ether.
B. Prepare a saturated solution in the same solvent.
C. Transfer tissue from absolute alcohol to equal parts of alcohol and ether.
D. From alcohol and ether to thin celloidin.
E. From thin celloidin to thick celloidin.
F. From thick celloidin to paper mold on cork.
G. Into 70 per cent alcohol for three days to harden.
H. Cut section with knife flooded with 70 per cent alcohol.
I. Stain.
J. Dehydrate.
K. Clear.
L. Mount.

METHOD OF MAKING FROZEN SECTIONS. (Wright.)

1. Place tissue not over 5 mm. thick in 10 per cent formalin for two or more hours.
2. Wash in water.
3. Freeze with CO_2 or ether.
4. Cut frozen sections.
5. Float sections in normal salt solution and then on slide.
6. Cover section with cigarette paper, then with blotter moistened with 95 per cent alcohol. Press. Remove papers.
7. Cover with absolute alcohol thirty seconds. Drain.
8. Flow over the section and adjacent parts of the slide a thin solution of celloidin in equal parts of absolute alcohol and ether. Drain off immediately.* The celloidin should form a coating so thin as to be invisible.
9. Flood the slide with 95 per cent alcohol and then immerse in water. This hardens the film.
10. Stain.
11. Dehydrate.
12. Clear with oil origanum.
13. Mount in Canada balsam.

STAINING TISSUES IN SECTIONS.

1. Hem-alum and eosin.
2. Delafield's hematoxylin.
3. Eosin and thionin.
4. Carmine stains.
5. Weigert's fibrin stain.
6. Jenner's stain.
7. Van Giesen for connective tissues.

STAINING BACTERIA IN SECTIONS.

1. Gram's method.
2. Eosin and thionin.
3. Thionin.
4. Methylene blue.

STUDY OF TUMORS.

Classification.

Cut sections and study:

1. Myomata.
2. Neuromata.
3. Angiomata:
    - *a.* Simple.
    - *b.* Cavernous.
4. Lymphangiomata:
    - *a.* Simple.
    - *b.* Cavernous.
5. Fibromata.
6. Myxomata.
7. Lipomata.
8. Chondromata.
9. Osteomata.
10. Gliomata.
11. Sarcomata:
    - *a.* Round celled.
    - *b.* Lympho-sarcoma.
    - *c.* Alveolar.
    - *d.* Spindle celled.
    - *e.* Melano sarcoma.
    - *f.* Osteoid.
    - *g.* Psammomata.
    - *h.* Myeloid.
    - *i.* Angio sarcoma.
    - *j.* Endothelioma.
12. Papillomata.
13. Adenomata.
14. Carcinomata:
    - *a.* Acinous.
    - *b.* Epitheliomata.
15. Cystomata.

ANIMAL INOCULATIONS—

1. Subcutaneous method:
   - *a.* Liquids.
   - *b.* Solids.
2. Intravenous method.
3. Inoculation into lymphatic system.
4. Inoculation into serous cavities:
   - *a.* Peritoneum.
   - *b.* Pleural cavity.
   - *c.* Subdural space.
5. Inoculation into anterior chamber of eye.

OBSERVATION OF ANIMALS AFTER INOCULATION—

1. Temperature.
2. Loss of weight.
3. Peculiar position in cage.
4. Loss of appetite.
5. Condition of coat.
6. Condition of secretions, etc.

POST-MORTEM EXAMINATION OF ANIMALS—

1. Cultures from the organs.
2. Cultures from the fluids.
3. Preparation and staining of tissues.

INCINERATION OF DEAD ANIMALS AFTER EXAMINATION—

MAKING COLLODIUM SACKS—

Paris method.
Gelatin capsule method.
With glass support and gelatin (method of Grubbs & Francis).

BACILLUS COLI Migula, 1900. [*Bacterium coli commune* Escherich.]

- A. Distribution.
- B. Isolate from sewage.
- C. Characteristics:
  - 1. Motility.
  - 2. Gram's method.
  - 3. Nonliquefaction of gelatin.
  - 4. Indol.
  - 5. Gas production.
  - 6. Colonies, etc.
- D. Pathogenesis.
- E. Varieties.
- F. Preparation of its toxin.
- G. Pathogenicity.
- H. Study of blood and organs of infected animals.
- I. Significance in drinking water.
- J. Viability.

BACILLUS ANTHRACIS Koch, 1876.

- A. History.
- B. Distribution.
- C. Morphology:
  - 1. Involution forms.
  - 2. Asporogenous form.
- D. Staining.
- E. Cultural characteristics.
- F. Spores:
  - 1. Formation—
    - Relative to oxygen.
    - Relative to nutrient media.
    - Relative to temperature.
  - 2. Development into bacteria.
- G. Pathogenesis (mouse, guinea pig, and rabbit.)
  - *a*. Pneumonic.
  - *b*. Hemorrhagic.
  - *c*. Local.
- H. Examination of liver, blood, spleen, lungs, and other organs.
- I. Vaccination:
  - First vaccine—kills mice, not guinea pigs or rabbits.
  - Second vaccine—kills mice and guinea pigs, not rabbits.
  - Third vaccine—kills mice and guinea pigs and some rabbits.
- J. Disposal of anthrax carcasses:
  - *a*. Burial, isolated districts.
  - *b*. Burning.
  - *c*. Sulphuric acid.
  - *d*. Boiling.
- K. Human anthrax:
  - *a* Malignant pustule.
  - *b*. Wool sorter's disease.
  - *c*. Intestinal form.
- L. Viability.
- M. Disinfection for anthrax.

BACTERIUM DIPHTHERIÆ (Löffler) Migula. [*Klebs-Löffler Bacillus.*]

- A. History.
- B. Morphology—relation to clinical types.
- C. Staining:
  - 1. Löffler.
  - 2. Neisser.
  - 3. Roux.
  - 4. Weigert's fibrin method for tissues.
  - 5. Gram's stain.
- D. Biologic characteristics.
- E. Pathogenesis:
  - 1. Guinea pig.
  - 2. Rabbit.
  - 3. Kitten.
- F. Toxin production.
- G. Antitoxin production:
  - 1. Standardization.
  - 2. Prophylactic use.
  - 3. Curative use.
- H. Obtain tubes and swabs from the health department for diagnosis.
- I. Pseudo-diphtheria bacillus.
- K. Xerosis bacillus.
- L. Viability.
- M. Disinfection for diphtheria.

BACTERIA IN INFLAMMATION AND SUPPURATION—

Study :

A. *Micrococcus aureus* (Rosenbach, 1884) Migula, 1900. [*Staphylococcus pyogenes aureus* Rosenbach, 1884.]
B. *M. pyogenes* (Rosenbach, 1884) Migula, 1900. [*S. pyogenes albus* Rosenbach, 1884.]
C. *M. citreus* (Passet, 1885) Migula, 1900. [*S. pyogenes citreus* Passet, 1885.]
D. "*Staphylococcus epidermidis albus* Welch."
E. *M. gonorrhœæ* (Bumm, 1885) Flügge, 1886.
F. *Streptococcus pyogenes* Rosenbach, 1884.
G. "*Streptococcus erysipelatos* Rosenbach," 1884
H. *Pseudomonas aeruginosa* (Schröter, 1872) Migula, 1900. [*Bacterium aeruginosum* Schröter, 1872 ; *Bacillus pyocyaneus* Gessard, 1882.]

Collect pus and inflammatory exudates from various sources, plate out and study bacteria found.

Study pus in smears on slides.

Ducrey's bacillus—Soft chancre.

Micrococcus melitensis Bruce, 1893.

THE TYPHOID GROUP—

Study :

A. *Bacillus typhosus* Migula, 1900.
B. *Bacillus dysenteriæ* Shiga, 1898 [not Migula, 1900].
C. *Bacillus enteritidis* Gaertner, 1888.
D. Para-colon bacillus.
E. Para-typhoid bacillus.
F. *Bacillus murium* Migula, 1900. [*Bacillus typi murium* Löffler, 1893.]
G. Bacillus of Danyz's virus.
H. *Bacillus psitticosis.*
I. *Bacillus icteroides* Sanarelli, 1897.
J. *Bacillus coli* Migula, 1900. [*Bacterium coli commune* Escherich.]
K. Methods of introduction into the system.
L. Channels of elimination.
M. Viability.
N. Disinfection.
O. Relation to the water supply.
P. Relation to flies and other insects.
Q. Widal reaction.—
  a. With hanging drop.
  b. In pipettes.

THE TUBERCLE GROUP—

- A. *Bacillus tuberculosis* Koch, 1882:
  - 1. Human. Isolate in pure culture from sputum.
  - 2. Bovine.
  - 3. Avian.
  - Tuberculin—
    - Production.
    - Uses:
      - 1. Diagnostic.
      - 2. Therapeutic.
- B. Acid proof organisms, resembling *B. tuberculosis:*
  - 1. Möller's grass bacillus. Isolate from timothy.
  - 2. Rabinowitsch's butter bacillus.
  - 3. Karlinsky's nasal secretion organism.
  - 4. Smegma bacillus.
- C. *Bacillus lepræ* Hansen.
- D. *Bacillus mallei* (Löffler, 1886) Migula, 1900:
  - Mallein.
  - Straus method in male guinea pig.
- E. Actinomycosis.
- F. Mycetoma, madura foot.
- G. Pathogenic streptothrices.

THE VIBRIO GROUP—

A. *Microspira comma* Schröter, 1886. [Koch's comma bacillus of Asiatic cholera.]
B. *Microspira Metshnikovi* (Gamaleia, 1888) Migula, 1900.
C. *Microspira Finkleri* Schröter.
D. Vibrio Denecke.
E. *Microspira aquatilis* (Günther, 1892) Migula, 1900.
  Pfeiffer's phenomenon.
  Widal reaction.
  Immunity.
  Haffkine's prophylactic.
  Viability.
  Disinfection for cholera.

RELAPSING FEVER.

BACILLUS PESTIS Kitasato & Yersin, 1894.

- A. Study—morphology, staining and cultural characteristics:
  - Viability.
  - Channels of entrance.
  - Channels of elimination.
  - Pathogenicity—guinea pigs, rabbits, rats, mice.
  - Diagnosis:
    - Yersin serum—
      - Method of manufacture and standardization.
      - Prophylactic use.
      - Curative use.
    - Haffkine prophylactic—
      - Methods of manufacture.
      - Uses.
- B. Disinfection.
- C. Quarantine.
- D. Relation of rats and other mammals to spread of plague.
- E. Relation of fleas, flies, and insects to spread of plague.

SEPTICEMIC GROUP—

A. Mouse septicemia.
B. Rouget du porc.
C. Chicken cholera.
D. Swine plague.
E. Ferret, duck, buffalo, and other plagues.
F. *Bacillus pestis* Kitasato & Yersin, 1894.
G. *Bacterium pneumoniæ* (Weichselbaum, 1886) Migula, 1900. [*Diplococcus pneumoniæ* Weichselbaum, 1886.]
H. *Bacterium pneumonicum* Migula, 1900. [*Pneumoniecoccus* Friedländer, 1882.]
I. *Bacterium influenzæ* Lehmann & Neumann, 1896.

ANAËROBIC TECHNIQUE—

- A. Kipps' hydrogen apparatus:
  - 1. Mechanics.
  - 2. Chemicals.
- B. Simple hydrogen generators.
- C. Solutions for purifying gas:
  - 1. Silver nitrate for chlorine.
  - 2. Potassium permanganate for oxygen.
  - 3. Lead acetate for sulphides.
- D. Media best suited for anaërobes:
  - 1. Freshly prepared media.
  - 2. Glucose media.
- E. Special methods:
  - 1. Fraenkel's method.
  - 2. Esmarch's method.
  - 3. Liborius' method.
  - 4. Buchner's method.
  - 5. Novy's jars.
  - 6. Wright's method.
  - 7. Pasteur Institute method.
  - 8. Vignal's tubes.
  - 9. Full tubes of bouillon.
  - 10. Deep stabs.
  - 11. Oil method.

ANAËROBES—

- A. *Bacillus tetani*, its toxin and antitoxin.
- B. *Bacillus œdematis* of malignant edema.
- C. *Bacillus Chauvæi* Arloing, Cornevin & Thomas, of symptomatic anthrax :
  - Vaccine—
    - 1. Manufacture.
    - 2. Use.
- D. *Bacillus Welchii* Migula, 1900. [*Bacillus aerogenes capsulatus* Welch & Nuttall, 1892.]
  - Disinfection for above.

PATHOGENIC MOLDS—
- *Ring worm:*
  - *Microsporon Audouini* (Gruby).
  - *Trichophyton*—
    1. *T. endothrix.*
    2. *T. ectothrix.*
  - Hair lesions.
  - Pure cultures.
- Favus:
  - *Achorion Schoenleinii* Remak.
    - In the hair and scale.
    - In pure culture.

YEASTS—

- *Saccharomyces cerevisiæ* Meyen (beer yeast) in pure cultures:
  - Effect on saccharine media.
  - Budding.
- Red yeast—*S. glutinis.*
- Black yeast—*S. niger.*
- *Saccharomyces albicans* (Robin) Reess, 1877 [*Oidium albicans* Robin] thrush, in pure culture.
- Pathogenic yeasts.

EXAMINATION OF URINE—

Note:

1. Odor.
2. Color.
3. Deposit. Filtration of specimen.
4. Specific gravity.
5. Reaction.
6. Quantity in twenty-four hours.
7. Albumen—

   Tests for albumen; quantitative and qualitative:
   - *a.* Heat (phosphates).
   - *b.* Nitric acid, in test tube and in glass.
   - *c.* Ferro-cyanide potash; acetic acid.
   - *d.* Picric acid, Esbach's albumenometer.
8. Sugar—

   Test for sugar:
   - *a.* Fehling's solution.
   - *b.* Bismuth.
   - *c.* Quantitative estimation with yeast and with Fehling's solution.
9. Estimation of urea.
10. Bile, test for.
11. Microscopic examination of sediment for bacteria, casts, crystals, blood, etc., *Schistosoma hæmatobium* (Bilharz, 1852) Weinland, 1858.
12. Test for chlorides.
13. Diazo reaction.
14. Bacteriologic examination of urine.
15. Disinfection of urine.

BACTERIOLOGIC AND MICROSCOPIC EXAMINATION OF FECES—

1. Microscopic:
   - *a.* Normal constituents.
   - *b.* Intestinal parasites and their eggs—
     - *Ascaris lumbricoides* (Linnæus, 1758).
     - *Oxyuris vermicularis* (Linnæus, 1758) Bremser, 1819.
     - *Trichuris trichiura* (Linnæus, 1771).
     - *Uncinaria duodenalis* (Dubini, 1843).
     - *Uncinaria americana* Stiles, 1902.
     - *Tænia saginata* Gœze, 1782.
     - *Tænia solium* Linnæus, 1758.
     - *Dibothriocephalus latus* (Linnæus, 1758).
     - *Amœba coli* Lœsch, 1875.
2. Bacteriologic:
   - *a.* Normal flora.
   - *b.* Pathogenic—
     - *Bacillus dysenteriæ.*
     - *Bacillus tuberculosis.*
     - *Bacillus typhosus.*
     - *Microspira comma.*
3. Disinfection of feces.

BACTERIOLOGIC AND MICROSCOPIC EXAMINATION OF—

- **Sputum, nasal and other mucous secretions:**
  - Tubercle bacilli.
  - Pneumococci.
  - Influenza bacterium.
- Disinfection of sputum.

BLOOD.

Technique of:

1. Collecting blood for microscopic examination.
2. Collecting blood for bacteriologic examination—
   - *a.* Small amount.
   - *b.* Large quantities.
   - Of man.
   - Of other mammals.
   - Of birds.
   - Of reptiles.
3. Observe circulation of blood under microscope.
   - Mesentery of frog.
4. Estimate alkalinity.
5. Estimate specific gravity of blood with mixture of chloroform and benzol.
6. Examine fresh blood in hanging drop.
7. Examine fresh blood between slide and cover slip.
8. Study of blood films:
   - *a.* Method with cover glass.
   - *b.* Method with special rod.
   - *c.* Method with cigarette paper.
9. Fixation of blood films:
   - *a.* Heat, 150° C. for about five minutes.
   - *b.* Alcohol and ether.
   - *c.* Formalin.
   - *d.* Formaldehyd.
   - *e.* Acetic acid bichlorid.
10. Staining.
    - *a.* Ehrlich-Biondi-tri-acid stain.
    - *b.* Jenner's stain.
    - *c.* Romanowsky's stain.
    - *d.* Goldhorn's fluid.
    - *e.* Eosin and methylen blue.
    - *f.* Thionin and eosin.
11. Counting red corpuscles.
    - Thoma-Zeiss hemocytometer.
12. Estimating the number of leucocytes:
    - *a.* With the Thoma-Zeiss hemocytometer.
    - *b.* Comparative count of—
    - *a.* Small lymphocytes.
    - *b.* Large lymphocytes and transitional forms.
    - *c.* Polymorphonuclear neutrophiles.
    - *d.* Eosinophiles.
    - *e.* Mast cells.
13. Estimating the amount of hemoglobin:
    - With—
    - *a.* Dare's hemoglobinometer.
    - *b.* Oliver's hemoglobinometer.
    - *c.* Gower's hemoglobinometer.

BACTERIA IN THE AIR—

1. Number:
   - Sewer air.
   - Expired air.
   - Sea air.
   - City air.
   - Country air.
   - Air in houses, hospitals, etc.—
   - Relation to the sun.
   - Relation to habitation.
   - Relation to winds and traffic.
   - Relation to altitude.
   - Pasteur's method.
   - Miquel's method.
   - Aeroscopes.
   - Frankland's method.
   - Petri's method.
   - Hesse's method.
   - Straus' method.
2. Varieties.
3. Estimation of ammonia in the air.
4. Estimation of carbon dioxid in the air

BACTERIA IN WATER—

1. Count number per 1,000 c. c.:
   - *a.* In tap water.
   - *b.* In river water.
   - *c.* In spring water.
   - *d.* In rain water.
   - *e.* In ice.
2. Varieties.
3. Collecting samples.
4. Estimation of organic matter—
   - *a.* Free ammonia.
   - *b.* Albuminoid ammonia.
5. Estimation of nitrates and nitrites.
6. Estimation of chlorids.
7. Purification of water:
   - Heat.
   - Filters—
     - *a.* Household filters.
     - *b.* Field and camp filters.
     - *c.* Sand filters—
   - Alum.
   - Hyperoxid of chlorin.
   - Ozone.
   - Permanganate of potash.

BACTERIA IN SOIL—

1. Number.
2. Varieties.
   Nitrification.

PURIFICATION AND DISPOSAL OF SEWAGE.

1. Dilution.

This method is allowable for cities situated on the sea or on rivers whose flow is very large as compared with the sewage (100 : 1) and the unfiltered water of which is not used as a water supply.

2. Sewage farming.
3. Chemical precipitation.
4. Intermittent filtration.
   Needs sandy soil.
5. Septic tank.
6. Contact bed treatment.
7. Continuous filtration:
   - *a.* Scott-Moncrieff system.
   - *b.* The Ducat.
   - *c.* Whitaker & Bryant.
   - *d.* F. Stoddart.
   - *e.* G. Salford.

MILK—

1. Histology.
2. Bacteria in milk:
   Number.
   Origin.
   Varieties.
   Diseases conveyed by milk.
3. Fermentation—
   Lactic acid.
   Butyric acid.
   Sweet curdling.
   Abnormal.
4. Pasteurization:
   Theory and practical uses.
   Changes produced in milk.
5. Count and study colonies in fresh milk and milk bought in open market.
   Pasteurize, then count and study colonies.
6. Sterilization of milk by heat: practical application and changes produced in milk.
7. Milk preservatives.

PRINCIPLES AND PRACTICE OF DISINFECTION AND STERILIZATION.

Physical agents.

1. Sunlight—
   - Expose spores of anthrax, subtilis, etc.
   - Expose nonspore-bearing organisms as typhoid, diphtheria, etc., to the sunlight in thin films and for penetration. Note intensity of the light, temperature, dryness, and other conditions. Plant in bouillon.
2. Electricity—
   - Expose slips and cultures to various currents.
   - Expose to electric light.
   - Expose to X-rays.
3. Dryness and dry heat—
   - Expose slips, or threads with spore-bearing and nonspore-bearing organisms to :
     - *a.* Dryness at room conditions.
     - *b.* Dry in incubator at 37° C.
     - *c.* Dry over sulphuric acid.
     - *d.* Dry heat at 60° C.,
100° C.,
150° C.,
for variable times.
     - *e.* Expose paper slips saturated with—
       - *a.* Decomposing urine,
       - *b.* Sewage,
       - *c.* Garden earth,

       to 150° C. for one hour to test power of sterilization of this temperature. Hot-air sterilizer.

   NOTE.—Plant in bouillon and keep one week for growth.

5. Moist heat—hot and boiling water and steam :
   - Expose typhoid, diphtheria, and other nonspore-bearing organisms to 50°, 60°, 70°, 80°, and 100° C. moist heat for variable times ; plant in bouillon.
   - Expose anthrax, subtilis, and other spores to 70°, 80°, 90°, and 100° C. moist heat for variable times ; plant in bouillon.
6. Theory, mechanics, and practical application of steam in—
   - *a.* Koch steamer.
   - *b.* Arnold steam sterilizer.
   - *c.* Autoclave (steam under pressure).
   - *d.* Steam chamber (steam under pressure).

   Make practical tests in each.

TESTING OF ANTISEPTICS—

If the substance belongs to the Cresol group, prepare 1 per cent, 2 per cent, 3 per cent, 5 per cent, 7 per cent, 9 per cent, 10 per cent solutions of the substance in a set of bouillon tubes.

If the substance belongs to the metallic salts, make $\frac{1}{5000}$, $\frac{1}{3000}$, $\frac{1}{2000}$, $\frac{1}{1000}$, $\frac{1}{250}$, etc., solutions in a set of bouillon tubes.

Inoculate with :

a. Decomposing urine.
b. Sewage.
c. Garden earth.
d. Typhoid.
e. Pus cocci.
f. Anthrax spores, etc.

Incubate and make report on the following plan :

Name of substance.—Strength of solution.	Minutes.							
	1.	2.	3.	5.	10.	15.	20.	30.
Sewage	—	—	—	....	....	....	....	....
Garden earth	—	+	+	....	....	....	....	....
Decomposing urine..........................	—	—	+	....	....	....	....	....

— means no growth, + means growth.

TESTING GERMICIDES—

Saturate silk threads,
paper slips,
glass slips, etc., with
subtilis spores,
anthrax spores,
typhoid bacillus,
diphtheria bacillus,
pus cocci, etc.

Expose to the solutions of germicide in varying dilutions and for varying times at constant temperature, and at the same dilution at varying temperatures.

Wash or neutralize the germicide.

Plant in bouillon.

Incubate.

Report growth upon similar table to antiseptics.

Test the following upon the above plan—

A. Bichloride of mercury.
A. Carbolic acid and cresols.
C. Lime.
D. Copper and iron sulphates.
E. Formalin.

GASEOUS DISINFECTION—

- A. Formaldehyd:
  - *a.* Chemistry and physics.
  - *b.* Sheet method.
  - *c.* Generator—
    - 1. Kuhn lamp.
  - *d.* Regenerators without pressure—
    - 1. Trenner-Lee.
    - 2. Lentz.
  - *e.* Regenerators with pressure—
    - 1. Autoclave.
  - *f.* Heating paraform.
  - *g.* Spraying.
- B. Sulphur dioxid (SO_2):
  - *a.* Liquid sulphur dioxid.
  - *b.* Burning sulphur, pot method.
  - *c.* Sulphur furnace.
- C. Hydrocyanic acid gas.
- D. Chlorin gas.
- E. Ozone and oxygen.

INSECTICIDES—

A. Tobacco smoke.
B. Pyrethrum.
C. Hydrocyanic acid.
D. Formaldehyd gas.
E. Sulphur dioxide.
F. Coal oil.
G. Arsenical compounds.
H. Phosphorus.
I. Cobalt salts.
J. Carbon bisulphid for mosquitoes, adults, pupæ, and larvæ :
  Ants,
  Mosquitoes,
  Roaches,
  Fleas,
  Flies, etc.

1. THE DISINFECTION OF HOUSES, SHIPS, AND OBJECTS.
2. THE DISINFECTION AND PROPHYLAXIS AGAINST THE COMMUNICABLE DISEASES, ESPECIALLY:
    - *a.* Yellow fever.
    - *b.* Cholera.
    - *c.* Plague.
    - *d.* Smallpox.
    - *e.* Tuberculosis.
    - *f.* Typhoid fever.
    - *g.* Malaria.
    - *h.* Dysentery.
    - *i.* Diphtheria.
    - *j.* Pneumonia.
    - *k.* Leprosy.
    - *l.* Anthrax.
    - *m.* Tetanus.
    - *n.* Dengue.
    - *o.* Relapsing fever.
    - *p.* Typhus fever
    - *q.* Scarlet fever.
    - *r.* Measles.

Including channels of introduction and elimination.
Viability.
Disinfection.
Prophylaxis.
Management of epidemics.
Quarantine.

RABIES—

Study in dogs.
Study in rabbits.
Prepare spinal cords for preventive inoculation.
Principles and methods of Pasteur treatment.

VACCINATION (*vaccinia*)—

A. History.

B. Methods of production:

Vaccine lymph.

Vaccine pulp—

Dry points.

Glycerinated pulp.

C. Technique of vaccination.

D. Immunity produced.

E. Bacteriologic and microscopic study of vaccine matter.

F. Bodies in epithelial cells—cornea of rabbits.

Examination of vaccine—

G. Note the name of maker, kind of virus, etc.

H. Count the number of colonies upon:

1. Three glycerinated tubes upon agar.
2. Three glycerinated tubes or points upon gelatin.
3. Three dry points upon agar.
4. Three dry points upon gelatin.

I. Study the character of the organisms found upon media and animals.

J. Plant the entire contents of 6 tubes in bouillon, grow anaërobically and study the characters of the growth upon media and by inoculation in animals.

K. Study the dry points as in J.

L. Inoculate the contents of 12 tubes and 12 points directly into mice, rats, and guinea pigs.

M. Keep full notes of all results.

N. Visit to the vaccine farm for practical study of manufacture of vaccine commercially.

MALARIA—
1. Diagnosis.
2. Blood technique.
3. Relation to mosquito:
    - *a.* Tertian.
    - *b.* Quartan.
    - *c.* Aestivo-autumnal.

Mosquitoes—
Anatomy—dissections fresh specimens.
Sections in paraffin.
Development and raising.
Classification.
Diagnosis, especially:
- a. *Culex.*
- b. *Anopheles.*
- c. *Stegomyia.*

A. Malaria—the Anopheles:
Study *Anopheles* one each day after biting a malarial case.
Tertian.
Quartan.
Estivo-autumnal.
B. Yellow fever and the mosquito.
C. Filaria and the mosquito.

INSECTS AS FACTORS IN TRANSMITTING DISEASE—

- The mosquito.
- The fly.
- The ant.
- The flea.
- The bedbug.
- The roach.
- The tick, etc.

Texas fever.

Spotted fever of Bitter Root Valley, Mont.

TRYPANOSOMES—

Inoculate rat with *Trypanosoma Lewisi.*
Dourine.
Surra.
Nagana.
Mal de caderas.

FILARIAL EMBRYOS (*"Filaria sanguinis hominis"*) in the blood.

Filaria Bancrofti Cobbold, 1877.
Filaria diurna Manson, 1891.
Filaria perstans Manson, 1891.
Filaria Demarquayi Manson,, 1805.
Dracunculus medinensis (Linnaeus, 1758). [Guinea Worm.]

FERMENTS—
- Organized.
- Unorganized—enzymes.
- A. *Amylolytic:*
  - Ptyalin.
  - Diastase.
  - Amylopsin.
    - Convert starch and glycogen into sugar.
- B. ***Proteolytic:***
  - Pepsin.
  - Trypsin.
  - Bacterial peptonizing enzymes.
    - Convert proteids into peptones.
- C. *Steatolytic*—steapsin.
  - Split fats into fatty acids and glycerin.
- D. *Invertin*—in intestinal juice and yeast cells.
  - Convert cane sugar, maltose, lactose into glucose.
- E. *Coagulation:*
  - Rennet.
  - Fibrin ferment.
    - Convert soluble into insoluble ferments.
- F. *Alcoholic ferments:*
  - Saccharomyces.
    - Converts carbohydrates into alcohol, carbon dioxide, etc.

Fermentation in—
- Wine.
- Beer.
- Vinegar, acetic.
- Milk :
  - Lactic.
  - Butyric.
  - Koumys.
  - Kefir.

IMMUNITY—
- Natural.
- Artificial.
- Active.
- Passive.
- Acquired.

Serum therapy—
- Anti-infectious sera.
- Antitoxic sera.
- Prophylactic sera.
- Curative sera.

Ehrlich's side chain theory.

Phagocytosis.

Preventive vaccines—
- Vaccinia (smallpox).
- Rabies.
- Anthrax.
- Symptomatic anthrax.
- Swine plague.
- Hog cholera.
- Rouget du porc.
- Plague.
- Typhoid fever.
- Cholera.
- Tetanus.
- Diphtheria.

TRANSPORTATION OF DISEASED TISSUES AND BACTERIOLOGIC SPECIMENS, ETC., BY MAIL AND EXPRESS.

The Regulations of the Post-Office Department, Order No. 176, March 2, 1900.
Mailing cases.
Methods of sending dry specimens.
Methods of sending moist specimens.
Methods of preparing and forwarding samples for bacteriologic study.
Methods of preparing and forwarding samples for histologic study.